DTIC FILE COPY

U

SECURITY CLASSIFICATION OF THIS PAG

REP

AD-A223 756

Form Approved
OMB No 0704-0188

1a REPORT SECURITY CLASSIFICATION
U

RKINGS
N/A

2a SECURITY CLASSIFICATION AUTHORITY

3 DISTRIBUTION / AVAILABILITY OF REPORT
Distribution unlimited

2b DECLASSIFICATION / DOWNGRADING SCHEDULE
N/A

4 PERFORMING ORGANIZATION REPORT NUMBER(S)
University of California, Riverside

5 MONITORING ORGANIZATION REPORT NUMBER(S)
N/A

6a NAME OF PERFORMING ORGANIZATION
UC Riverside

6b OFFICE SYMBOL (If applicable)
ONR

7a NAME OF MONITORING ORGANIZATION
Office of Naval Research

6c ADDRESS (City, State, and ZIP Code)
Department of Chemistry
University of California
Riverside, CA 92521

7b ADDRESS (City, State, and ZIP Code)
800 North Quincy Street
Arlington, VA 22217-5000

8a NAME OF FUNDING / SPONSORING ORGANIZATION
Office of Naval Research

8b OFFICE SYMBOL (If applicable)
ONR

9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
N00014-89-J-3071

8c ADDRESS (City, State, and ZIP Code)
800 North Quincy Street
Arlington, VA 22217-5000

10 SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO	PROJECT NO	TASK NO	WORK UNIT ACCESSION NO
61153N	RR04108	4414913	

11 TITLE (Include Security Classification)
Chemical Modification of the Olfactory Receptor Epithelium of Vertebrate Species

12 PERSONAL AUTHOR(S)
Thomas Hellman Morton

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Annual	FROM 7/89 TO 6/90	6/28/90	16

16 SUPPLEMENTARY NOTATION
N/A

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
08		

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Olfaction, covalent modification, organic chemistry, Jeffamine, hydrophilic tethers, capillary electrophoresis

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

This project seeks to develop methodologies for attachment of cell-surface binding sites to solid surfaces using hydrophilic tethers that incorporate reversible linkers. Characterization of Jeffamine copolymers by capillary zone electrophoresis allows the chainlength distribution to be assayed for industrially available hydrophilic tether components. Organic synthesis is used to prepare shorter tether constituents with fluoridolyzable linkages.

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS

21 ABSTRACT SECURITY CLASSIFICATION
U

22a NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code)	22c OFFICE SYMBOL
Igor Vodyanoy	(202) 696-3945	ONR

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

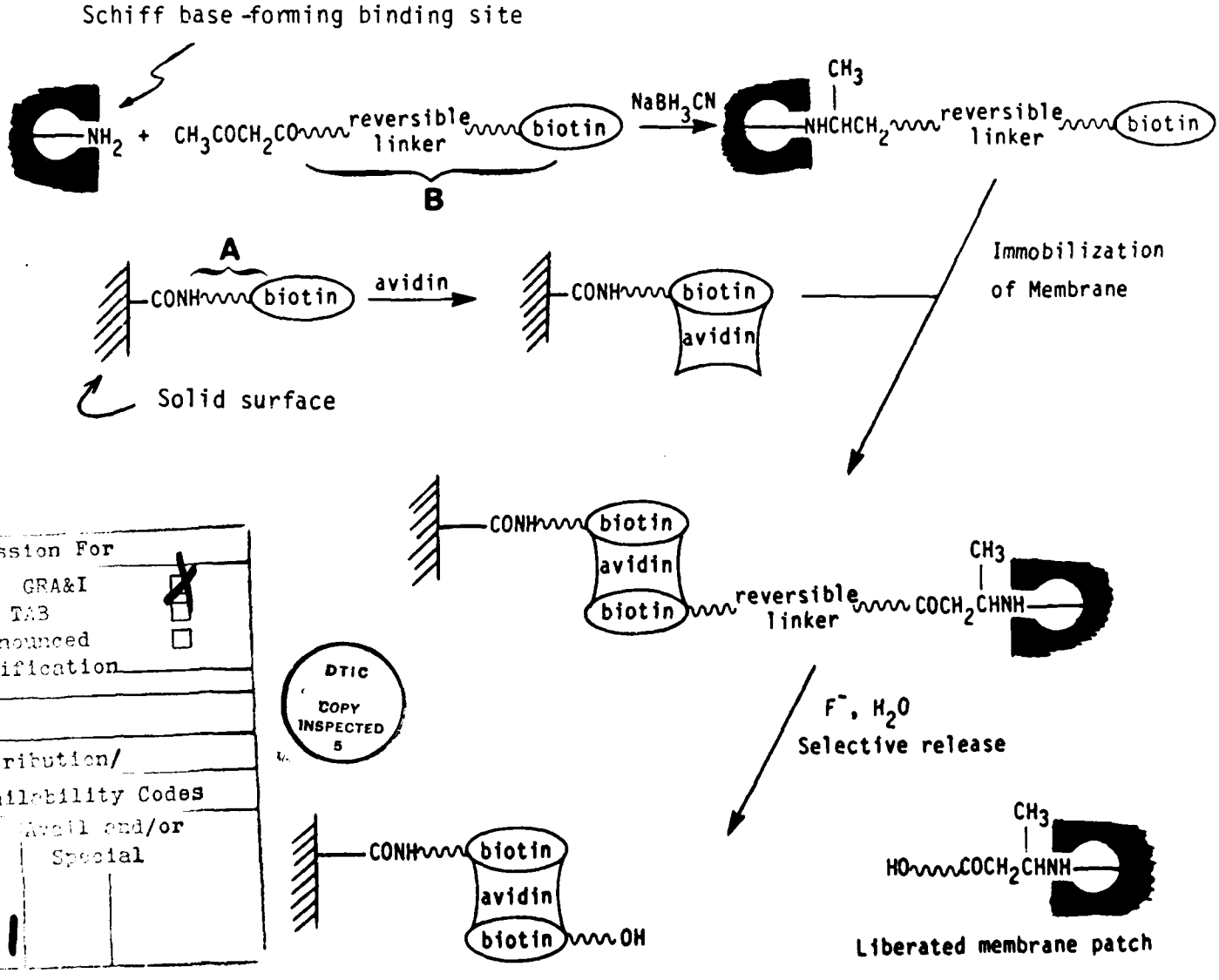
DTIC ELECTE JUL 03 1990 D

90 07 2

INTRODUCTION

The object of this investigation is to develop a methodology for covalent-attachment of cell-surface binding sites to solid surfaces, from which the sites may subsequently be selectively removed without denaturing them. The general strategy is represented by Scheme I, where application to a Schiff base-forming site imbedded in a membrane patch is illustrated. The pertinence of such sites to olfaction in vertebrate species (tiger salamanders, European starlings) has been demonstrated by a series of studies from this laboratory [1].

Scheme I



The tactics for membrane-to-surface linking under physiological, non-denaturing conditions involve forging the link via biotin-avidin binding. Two essential tether elements are required: A to connect a biotin moiety to the

surface and **B** to connect a biotin moiety to the membrane binding site.

The tethers **A** and **B** must both be hydrophilic, so that they will be fully extended in aqueous media and not tend to insert themselves into the membrane or coil up onto the surface. Other features differ somewhat between **A** and **B**. **A** must be long (>30Å when fully extended) so that the membrane not be adsorbed onto the surface, but is need not be chemically homogeneous. **B** may be shorter, but must be chemically homogeneous and incorporate a reversible linker that permits removal of the surface-bound membrane patches under sufficiently mild conditions that they can be recovered intact.

This report describes synthesis/characterization of the tether elements **A** and **B** and is therefore divided into two parts. The first part presents the characterization of Jeffamine, a commercially available copolymer that is suitable for tether **A**. Characterization of the distribution of chainlengths is necessary, for if **A** is not chemically homogeneous its dispersion must be known for it to be used. This characterization has involved the development of new analytical methodology using capillary zone electrophoresis (CZE) and was performed by Dr. L. Amankawa and Prof. W.G. Kuhr. The synthesis of tether element **B** and its characterization was performed by Mr. W.C. Lin and Prof. T.H. Morton.

A. CHARACTERIZATION OF JEFFAMINE CHAINLENGTH DISTRIBUTION

The oligomer distribution of several polyoxyalkylene diamine (Jeffamine) polymers, ranging in molecular weight from 600 to 2000 has been characterized by pre-column derivatization and capillary zone electrophoresis / fluorescence detection. Separation mechanism is solely based on the differences in the electrophoretic mobilities of the oligomer units. The effect of the pre-column derivatization reaction on oligomer separation is also presented. Oligomer separation is only possible for a 1:1 (sample:derivatizing agent) derivatized sample. It is possible by this method to separate 30 oligomers with an average molecular weight of 600 mass units, and which differ in size by only a methylene unit in less than 15 minutes with a separation efficiency of about 200,000 theoretical plates.

HPLC is one of the methods that is commonly used for separating and analyzing polymer composition [2-3]. Non-ionic surfactants containing different lengths of polyethylene oxide oligomers have been separated and characterized according to the number of ethylene oxide units by normal phase chromatography on an amino column. The separated components were monitored by both evaporative light scattering (ELS) and UV absorption detectors [2]. In the same report, alkyl ether sulfates and sulphonates were separated and characterized by reversed phase chromatography. Separations based on ion-exchange chromatography have also been widely used

for characterizing oligomeric distribution of polymer products [4-6]. Ernst [4] has separated phenol ethoxylate homologues with a Partisil PXS 10/25 SCX column and CH_3CN:THF:MeOH (25:25:50) as eluant.

High resolution supercritical fluid chromatography has also been used extensively for studying the oligomeric distribution of polymer products [7-9]. In one report [7], mixtures of alkyl ethoxylates were separated using CO_2 as mobile phase on a BP-10 column. Gel permeation chromatography [10-11], field-flow fractionation [12] and mass spectrometry [13-16] have all been used as analytical techniques for characterizing polymer products. Among all the available techniques, mass spectrometry is the only method that can provide both structural information as well as the oligomeric distribution of a particular material. Its application in polymer characterization is however, less common owing to the high cost of instrumentation.

In the past decade capillary zone electrophoresis (CZE) has developed into an efficient and fast analytical technique for the separation of complex mixtures such as amino acids, proteins, peptides, nucleotides and biopolymers [17-27]. In spite of the high efficiency separation capability of CZE there has only been one reported application [28] of this technique for the separation and analysis of other polymeric materials. In that study, polystyrene nanospheres were separated by CZE. The authors showed that it was possible to resolve polystyrene particles in the size range 39 nm to 683 nm. The separation mechanism was however, believed to involve differences in surfactant mediated particle-capillary wall interactions and not by differences in electrophoretic mobilities of the particles.

In this report we describe the application of CZE/fluorescence detection for the separation and characterization of the oligomeric distribution of two polyoxyalkylene diamine (Jeffamine-ED) polymers. These compounds have been suggested for use as antistatic and epoxy curing agents [29, 30]. The separation mechanism utilized in this study is based on the differential electrophoretic mobilities of the protonated oligomers in the presence of an applied electric field. Eluted components (oligomers) are detected by monitoring the fluorescence emission of a fluorescent label (2,3-naphthalenedialdehyde, NDA) attached by pre-column derivatization of each oligomer in the sample prior to separation. The effect of the degree of derivatization of the sample with the fluorescent label and the pH of the electrophoresis buffer on oligomer resolution will be discussed. Finally, structural information on each separated oligomer will be obtained by comparing the oligomer distribution obtained by CZE with the distribution independently obtained by Fourier transform mass spectrometry (FTMS) [15].

Experimental

Apparatus

The instrument used in this work is a commercial electrophoresis instrument (Model 270A, Applied Biosystems, Inc., San Jose, CA), which was loaned to Prof. W.G. Kuhr by the manufacturer. It is microprocessor controlled and consists of an auto sampler, a vacuum injection system, an electromigration system, a thermostated (30-60°C) capillary compartment and a UV-vis absorbance detector [31,32]. Additionally, this instrument is equipped with a prototype fluorescence detector, including a 75W xenon arc lamp, powered by an auxiliary power supply (Model No. C2177-01, Hamamatsu, Japan), mounted in standard configuration. Excitation wavelengths between 190 nm and 700 nm were selected via the inbuilt monochrometer. The detector cell consists of a 0.5-1.0 cm window on the capillary where the polyimide coating has been removed. This optical window is mounted in an assembly where the excitation light is focused radially by a spherical sapphire lens into the center of the capillary so that the intensity of the light passing through the solution is maximized. The fluorescence emission is collected at right angles to the plane of the source beam and the capillary by two fiber optics, one on each side of the detector cell. Light from these fiber optics is filtered through a manually interchangeable 475nm longpass glass color filter and detected by a photomultiplier tube. The instrument can be operated in both manual and automatic modes.

Reagents

Water was distilled and deionized (Millipore, Bedford, MA). Methanol (Fisher Scientific, Fair Lawn, NJ) was of analytical grade and was used without further purification. Stock buffer was composed of 0.1 M trisodium phosphate (Fisher Scientific), 0.2 M boric acid (Sigma Chemical Co., St. Louis) and 0.05 M citric acid (Fisher Scientific). The electrophoresis buffer was made by diluting the stock buffer with appropriate volumes of methanol and water such that the final concentration of the buffer components are half that of the stock buffer at the appropriate pH [33] and also contain 20% of methanol (v/v). The final buffer was filtered through a 0.45 μm filter prior to use.

Stock sample solutions (10.0 mM) were prepared by weighing appropriate amounts of Jeffamine (Texaco Chemical Co., Bellaire, TX) and dissolving in 10.00 mL of water. These solutions were stable and hence were prepared weekly. NDA derivatizing reagent (10.0 mM) was prepared fresh daily by dissolving appropriate weights of NDA (Molecular Probes, Inc., Eugene, OR) in methanol. A stock solution (0.10 M) of NaCN (Mallinckrodt, Inc., Paris, KY) was prepared in water.

Separation capillary: The fused silica capillaries used with the Applied Biosystems Instrument was 75 cm long (60 cm to the detector), 50 μm i.d and 370 μm o.d. Prior to use, the capillary was cleaned with 1.0 M NaOH for 10 min. followed by a 10 min rinse with water before filling with the electrophoresis buffer. Cleaning was done by connecting one end of the capillary to a vacuum line (< 10 torr) while the other end was immersed in the appropriate solution.

Pre-column Derivatization Procedure: 1.0 mL of the Jeffamine solution was mixed with 1.0 mL of NaCN, 5.0 mL of phosphate buffer pH 9.5 followed by 1.0 mL of the NDA derivatizing reagent. The mixture was allowed to stand for 15 minutes after which 2.0 mL was pipetted into 5.0 mL of methanol and then made up to 15.0 mL with buffer. This gives a final sample concentration of 180 μM.

Results and Discussion

Sample

Jeffamines (polyoxyethylenediamines) are aliphatic primary diamines structurally derived from propylene oxide-capped polyethylene glycol and have the general formula:

$$NH_2CH_2CH(CH_3) - [OCH(CH_3)CH_2]_{m-a} - [OCH_2CH_2]_n - [OCH_2CH(CH_3)]_a - NH_2$$

Each oligomer is composed of polypropylene glycol (PPG) and polyethylene glycol (PEG) hence for simplicity they are described by the empirical formula $R(PPG)_m(PEG)_n$, where $R = C_3H_{10}N_2$. The subscripts m and n are the number of PPG's and PEG's respectively in the oligomer. Like most polymeric materials, the commercially available Jeffamines are composed of mixtures of oligomers of different chemical composition and molecular weight. These products are therefore known generically as Jeffamine ED-X, where X is the average molecular weight.

The Jeffamine ED compounds are water soluble and have been suggested a starting a material for the synthesis of durable antistatic agents [28], and also as epoxy curing agents [29]. Oligomer composition and distribution of Jeffamine ED-600, Jeffamine ED-900 and Jeffamine ED-2001 have been characterized previously by laser desorption Fourier transform mass spectrometry [15]. Four different oligomer compositions are apparent from the mass spectra (not shown). These series are $[R(PPG)_1(PEG)_n + H]^+$, $[R(PPG)_2(PEG)_n + H]^+$, $[R(PPG)_3(PEG)_n + H]^+$ and $[R(PPG)_4(PEG)_n + H]^+$. For each series a variable number of PEG's ($3 \leq n \leq 12$) are associated with the same number of PPG's. Oligomers in the same series are separated in weight by 44 amu (the weight of an ethylene oxide unit) while between series oligomers are separated in weight by 58 amu (the weight of a propylene oxide unit). In consequence, the entire mass spectra consists of peaks at m/z numbers that

are separated by either 14 a.m.u's or 2 a.m.u's. Peaks that are separated by 2 a.m.u result from peaks from the series $[R(PPG)_1(PEG)_n + H]^+$ and $[R(PPG)_4(PEG)_n + H]^+$. It is apparent from the mass spectra that Jeffamine ED-600 is composed of a complex mixture of oligomers with molecular weights ranging from 300 to 800 mass units. Similar oligomer distribution has also been obtained for the other Jeffamine ED compounds [15]. It is this complex oligomer distribution of the Jeffamines that is being characterized by CZE.

Sample Preparation

The Jeffamines absorb weakly in the UV ($\varepsilon 256 = 24$) hence they cannot be detected in the UV with any high degree of sensitivity. In view of this, the sample was fluorescence-labeled by derivatizing it with 2,3-naphthalenedialdehyde (NDA) prior to separation. Details of the derivatization reaction has previously been described [34, 35]. In this work however, Jeffamine and NDA were reacted in a 1:1 mole ratio instead of the large excess of NDA commonly used. This derivatization procedure was necessary in order to only derivatize one of the two primary amino groups on the Jeffamine molecule. The significance of this requirement is discussed in the following section. The UV absorbance and emission spectra of the derivatized Jeffamines are shown in Figure 1.

CZE Separation

The separation mechanism utilized for separating the components (oligomers) of Jeffamine is based on the differential mobilities of charged species under electrophoretic conditions. Although the pKa of Jeffamine was not determined in this work, it is expected to be high ($pKa \geq 10$) [36] because of the presence of the two primary amino groups. In low pH buffers ($pH \leq 10$) the components of Jeffamine will exist in the protonated form and hence will migrate toward the cathode under the influence of an applied electric field. The rates of migration of these components will depend on their intrinsic electrophoretic mobilities, and the component with the smallest molecular weight will have the highest mobility and hence elute first.

In this study, sample preparation involved precolumn derivatization with NDA. The labeling reaction couples NDA to the sample molecule through the primary amino group. When Jeffamine is derivatized with excess NDA, the coupling occurs at both amino groups to yield a compound which contains two NDA molecules per each sample molecule. The derivatization reaction therefore results in the conversion of the primary amino nitrogen into a heterocyclic (benzisoindole) nitrogen. Consequently, the degree of dissociation of the sample is completely changed. Indole derivatives have a characteristic small pKa values (indole has pKa = -2.4 [36]), and therefore will not be protonated significantly above pH 1.00. Complete derivatization of Jeffamine with NDA

therefore negates the ability to protonate the sample components in normal electrophoretic buffers ($2 \leq pH \leq 10$). To ensure component separation by CZE, the derivatization procedure was modified so as to couple NDA to only one of the two primary amino groups on Jeffamine. The acid-base properties of the uncoupled primary amino group is therefore preserved to be utilized for CZE separation. Although detection by post-column derivatization has been suggested as an alternative detection procedure for avoiding problems associated with pre-column derivatization, such as the one discussed above, it was not used in this work since NDA decomposes rapidly in the presence of NaCN.

The electropherogram for a Jeffamine ED-600 sample that was derivatized by reacting one part of Jeffamine with one part of NDA is shown in Figure 2A (Buffer pH = 4.2). The multiple peaks at migration time about 12.0 min correspond to protonated oligomer peaks, while the peak a migration time of 22.2 min corresponds to co-elution of all components that were unprotonated in the running buffer. All the unprotonated components elute at the same time because their rate of elution is controlled only by the rate of electroosmotic flow. Resolution of the protonated oligomers is possible because of the differences in the values of their electrophoretic mobilities. It is also apparent from this figure that although the sample and the derivatizing reagent, NDA, were reacted in 1:1 mole ratio, a small fraction (~ 20%) of the sample was completely derivatized with two parts of NDA for each mole of the sample.

The effect of the extent of NDA derivatization on the separation of Jeffamines is demonstrated in Figure 2B. Jeffamine ED-600 was derivatized by reacting one part of the sample with three parts of NDA so as to ensure that NDA was coupled to both amino groups on Jeffamine. As expected there is a complete absence of peaks corresponding to protonated oligomer. The single peak in the electropherogram has the same elution time as the second peak in Figure 2A and therefore is composed entirely of neutral components. Figure 2C is the electropherogram of Jeffamine ED-2001 derivatized by reacting one part of the sample with an equivalent amount of NDA. The broad peak at elution time around 16.0 min corresponds to poorly resolved protonated oligomer ion peaks. Again the second peak corresponds to double derivatized components. The fact that the second peaks in all three figures have the same elution time confirms that these components are neutral and therefore elute only because of electroosmotic flow. These components therefore act as internal markers and hence their elution time can be used to calculate the electroosomotic mobility under our experimental conditions. Using an average elution time of 22.2 min the measured electroosmotic mobility is 1.3 x 10-4 cm2/V.S. This value agrees with a previously reported value obtained for a silica capillary at pH = 4.0 [23].

The effect of pH on the resolution of Jeffamine ED-600 that was derivatized with an equal amount of NDA is demonstrated in Figure 3. At pH = 11.3 (Figure 3A) there is absolutely no evidence of oligomer separation. This is not surprising since all the sample components, both singly and doubly labeled components, exist as neutral species and at this pH elute only because of electroosmotic flow. At pH = 9.3 (Figure 3B) there is discrimination between the singly and doubly labeled components. All the single labeled components are partially protonated and hence migrate at a rate controlled by the rates of both electroosmosis and electrophoresis. These components therefore migrate faster and consequently are eluted first. Resolution is however, poor because at this pH the main factor controlling elution rate is electroosmosis. The rate of electroosmotic flow decreases with decreasing pH so that at pH = 4.2 (Figure 3C) the small differences in the electrophoretic mobilities of the protonated components become significant resulting in better resolution. It is important to note that all components of this matrix have the same charge and the same overall structure. Thus, the separation is based solely on the differences in the mass/charge ratio of the oligomers.

B. SYNTHESIS OF A BIOTINYLATED TETHER CONTAINING A FLUORIDOLYZABLE LINKER

Covalent attachment of biotin to a cell-surface receptor via a reversible linker requires that the tether possess two features: (1) the tether must be hydrophilic and be chemically homogeneous; and (2) the tether must contain a linkage that is stable under physiological conditions, but which an be hydrolyzed at will under conditions that do not damage the binding site or its associated membrane patch.

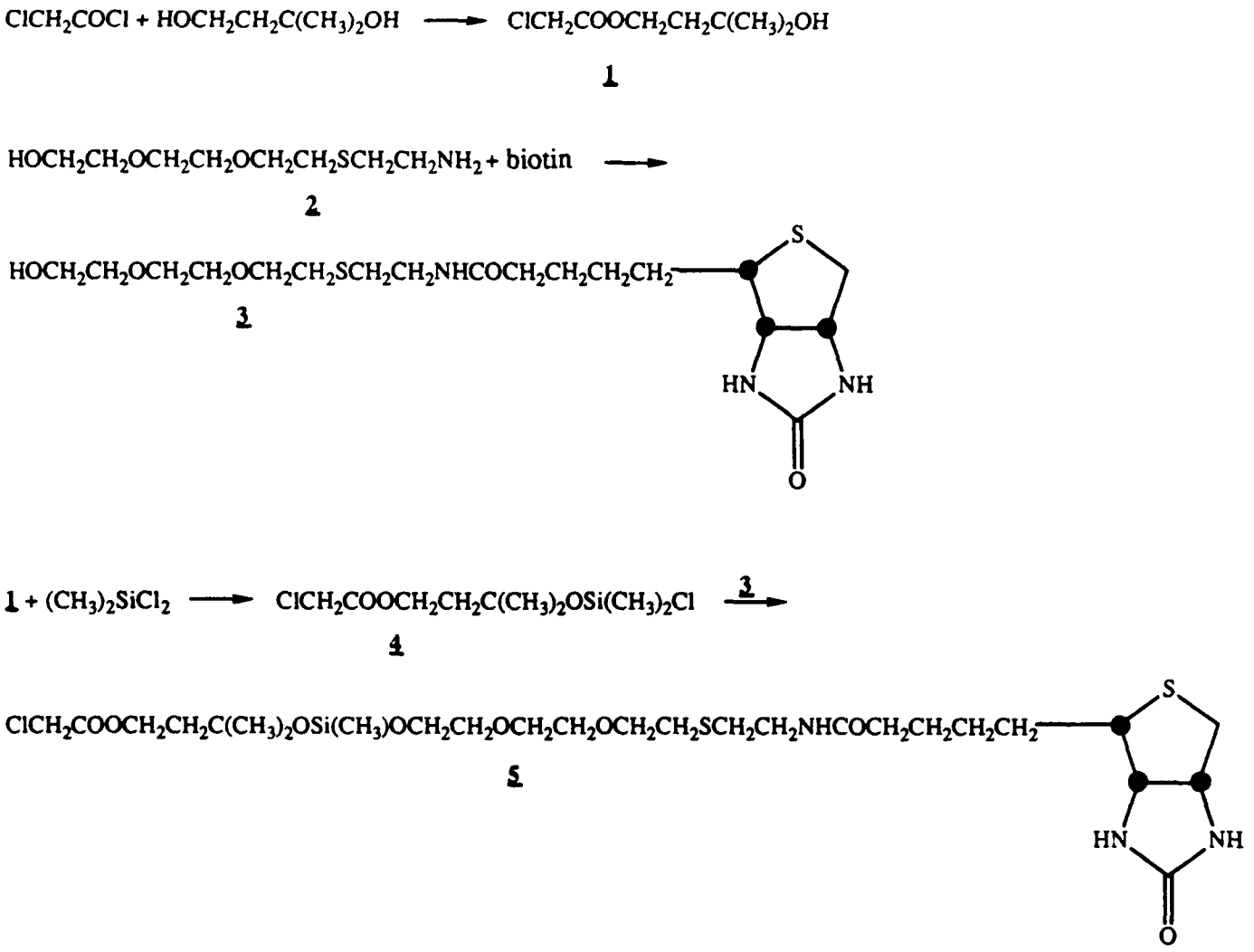
The appropriate tether has been constructed as shown in Scheme II. The reversible linker element is the $-C(CH_3)_2-O-Si(CH_3)_2-O-CH_2-$ portion of the hydrophilic chain, which we find to be stable in aqueous solution for days at physiological pH. In the presence of 0.5 M aqueous KF (which is otherwise non-destructive) one of the silicon-oxygen bonds ions rapidly hydrolyzes under the catalytic influence of the fluoride ion.

Experimental

Commercially available reagents were obtained from Aldrich Chemical Corp. and used without further purification unless otherwise specified. Pyridine was freshly distilled from CaH_2 prior to use. Proton and carbon NMR spectra were recorded on a General Electric QE300 instrument.

1-Chloroacetoxy-3-methyl-3-butanol (1). Chloroacetyl chloride (12.1 g, 10.7 mmole) in ether (200 mL) was added dropwise over a period of 6 hours to a magnetically stirred solution of 3-methyl-1,3-butanediol

Scheme II



(10.0 g, 96 mmole) and pyridine (8.5 g, 11 mmole) in ether (120 mL) at -15°C under nitrogen, after which the reaction mixture was allowed to warm to room temperature. The mixture was then filtered to remove precipitated pyridinium hydrochloride and washed with saturated aqueous $CuSO_4$ (10 mL) and twice with saturated aqueous NaCl (10 mL). The filtrate was dried by gravity filtration through anhydrous $NaSO_4$ and solvent removed under aspirator pressure on a rotary evaporator. The colorless product **1** (13.02 g, 54% yield) was distilled under reduced pressure (bp 90-91°C .05 Torr): ^1H NMR (300 MHz, $CDCl_3$) δ1.24 (s, 6H), 1.84 (t, J = 6.9 Hz, 2H), 2.21 (s, 1H), 4.03 (s, 2H), 4.34 (t, J = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) ppm 167.23, 69.73, 63.17, 41.18, 36.95, 29.52.

S-2-[2-(2-hydroxyethoxy)ethoxy]ethylcysteamine. Neat 2-[2-(2-chloroethoxy)ethoxy]ethanol (10.6 g, 62 mmole) was added at once to a solution of 2-aminoethanethiol hydrochloride (6.8 g, 60 mmole) and sodium bicarbonate (10.4 g, 124 mmole) in 160 mL 1:1 (v/v) aqueous 1,4-dioxane. The reaction mixture was refluxed for 48 hours with magnetic stirring, after which solvent was removed under aspirator pressure on a rotary evaporator. The residue was dissolved in $CHCl_3$ (100 mL) and filtered to remove inorganic salts. The filtrate was concentrated to a light-green liquid, which was distilled under reduced pressure. The colorless product **2** (7.1 g, 51% yield) was collected at 144-146°C under 0.10 Torr: ^1H NMR (300 MHz, $CDCl_3$) δ2.42 (bs, 3H), 2.68 (m, 4H), 2.87 (t, 2H), 3.63 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) ppm 72.97, 71.18, 70.32, 70.33, 61.40, 40.95, 37.08, 31.67; IR (in CCl_4) cm^{-1} 3606.9, 3308.1.

S-2-[2-(2-hydroxyethoxy)ethoxy]ethylcysteamine biotinamide. A 3-neck flask was charged with 1,1'-carbonyldiimidazole (3.3 g, 20 mmole) and biotin (4.9 g, 20 mmole) in 50 mL DMF under argon at room temperature. After 3 hours stirring neat **2** (4.48 g, 21 mmole) was added dropwise with magnetic stirring, and stirring was continued for 24 hours, after which the solvent was removed under reduced pressure with a vacuum pump. The residue was dissolved in 100 mL hot methanol, crystalized by adding 400 mL of ether (cooled with an ice bath) and recrystalized from t-butanol/hexane (7.4 g, 80% yield). ^1H NMR (300 MHz, $CDCl_3$) δ1.47 (q, 2H), 1.71 (m, 4H), 2.24 (t, J = 7.5 Hz, 2H), 2.75 (m, 5H), 2.93 (dd, 1H), 3.15 (q, 1H), 3.45 (q, 2H), 3.69 (m, 11H), 4.33 (m, 1H), 4.52 (m, 1H), 5.20 (s, 1H), 6.16 (s, 1H), 6.71 (t, J = 5.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) ppm 25.54, 28.04, 28.07, 31.55, 32.43, 34.66, 35.82, 38.69, 40.56, 55.47, 60.13, 61.58, 61.77, 70.279, 70.308, 70.99, 72.52, 163.64, 173.29.

S-2-[2-(2-(3-chloroacetoxy-1,1-dimethyl-1-propoxy)dimethylsiloxyethoxy)ethoxyethylcysteamine biotinamide. Dichlorodimethylsilane (.34 g, 2.6 mmole) was added to a solution of **1** (.43 g, 2.4 mmole) and imidazole (.18 g, 2.6 mmole) in DMF (3.0 mL) at room temperature under nitrogen and stirred for 12 hours. A solution of **3** (.84 g, 2.0 mmole) and imidazole (.20 g, 3.0 mmole) in DMF (6 mL) was then added dropwise to the reaction mixture. After 6 hours solvent was removed under reduced pressure with a vacuum pump and residues extracted with THF. After concentrating the THF solution flash chromatography with isopropanol/hexane (3/1) on silica gel was run to obtain product **5**: ^1H NMR (300 MHz, $CDCl_3$) δ.13 (s, 6H), 1.32 (s, 6H), 1.46 (m, 2H), 1.70 (m, 4H), 1.86 (t, J = 7.5 Hz, 2H), 2.23 (t, J = 7.5 Hz, 2H), 2.73 (m, 5H) 2.92 (dd, 1H), 3.16 (m, 1H), 3.44 (q, J = 6 Hz, 2H), 3.59 (t, J = 5.4 Hz, 2H), 3.64 (m, 6H), 3.81 (t, J = 5.4 Hz, 2H), 4.06 (s, 2H), 4.35 (m, 3H), 4.52 (m, 1H), 5.06 (s, 1H), 5.86 (s, 1H), 6.42 (t, J = 5.4 Hz, 1H).

Results and Discussion

Synthesis of tethered biotin **5** is achieved in two stages. First an excess of tertiary alcohol **1** is allowed to react with dichlorodimethylsilane. Because of steric hindrance only one mole of the tertiary alcohol displaces chloride, yielding tert-alkoxychlorodimethylsilane **4**, an intermediate which was not characterized except by its further reaction. Addition of one equivalent of the biotinylated primary alcohol **3** leads to displacement of the other chloride to yield the desired product **5**. The yield of this second step is very sensitive to traces of moisture, and we found that prior recrystallization of **3** from tert-butanol/hexane was necessary in order for the reaction to give a satisfactory yield. We believe that the hydrogen bond-accepting ability of the hydrophilic chain of **3** makes it virtually impossible to free it from hydroxylic impurities. If **3** contains traces of water, that impurity intercepts **4**. Recrystallization from tert-butanol replaces this impurity with a tertiary alcohol, which does not react with **4**.

FIGURE LEGENDS

Figure 1. Fluorescence emission spectra of NDA derivatized Jeffamine ED-600. Sample concentration = 40 μM, Buffer pH = 4.2. The corresponding UV absorbance spectra is shown (insert).

Figure 2. Electropherograms showing the effects of single (1:1) and double (1:2) NDA derivatization on oligomer separation of Jeffamine ED polymers. Mole ratio of Jeffamine ED 600 to NDA reacted are: (A) 1:1; (B) 1:3; (C) Jeffamine ED 2001:NDA = 1:1. CZE parameters: Capillary, 75 cm (60 cm to detector), 50 μm i.d. Injection, 2 s of 180 μM sample at 15 KV; run, 25 KV; current, 15 μA, temperature, 30 °C. Instrument: Applied Biosystems, Inc., Model 270A. Xenon arc lamp at an excitation wavelength of 442 nm.

Figure 3. Electropherograms showing the effect of pH on oligomer separation of Jeffamine ED 600. CZE parameters: Capillary, 75 cm (60 cm to detector), 50 μm i.d. Injection, 2 s of 180 μM sample at 15 KV; run, 25 KV; temperature, 30 °C. (A) pH = 11.3, current = 62 μA; (B) pH = 9.3, current = 49 μA; (C) pH = 4.2, current = 15 μA. Instrument: Applied Biosystems, Inc., Model 270A. Xenon arc lamp at an excitation wavelength of 442 nm.

FIGURE 1

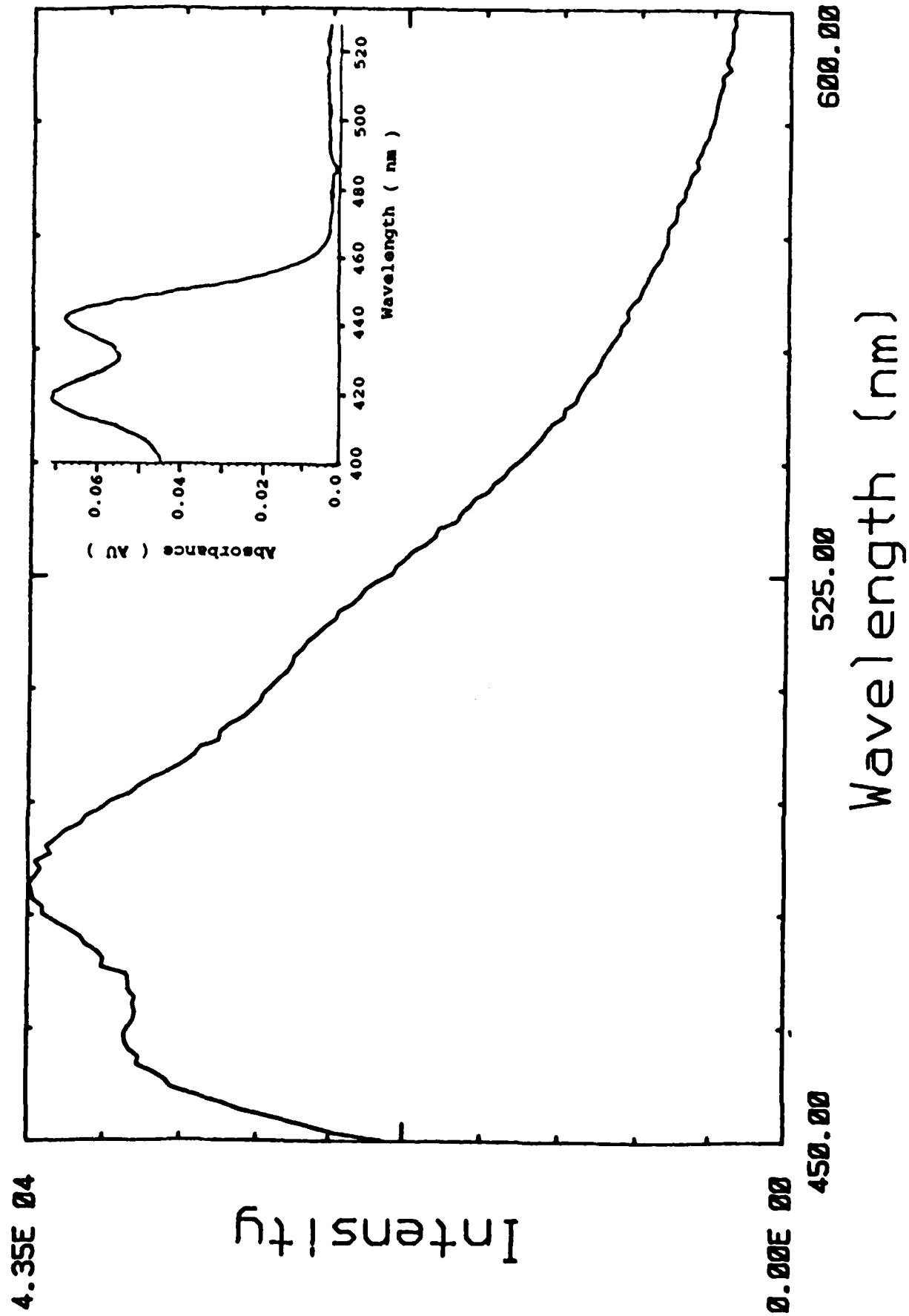

Intensity
4.35E 04
0.00E 00
450.00
525.00
600.00
Wavelength (nm)
Absorbance (AU)
0.06
0.04
0.02
0.0
400
420
440
460
480
500
520
Wavelength (nm)

FIGURE 2

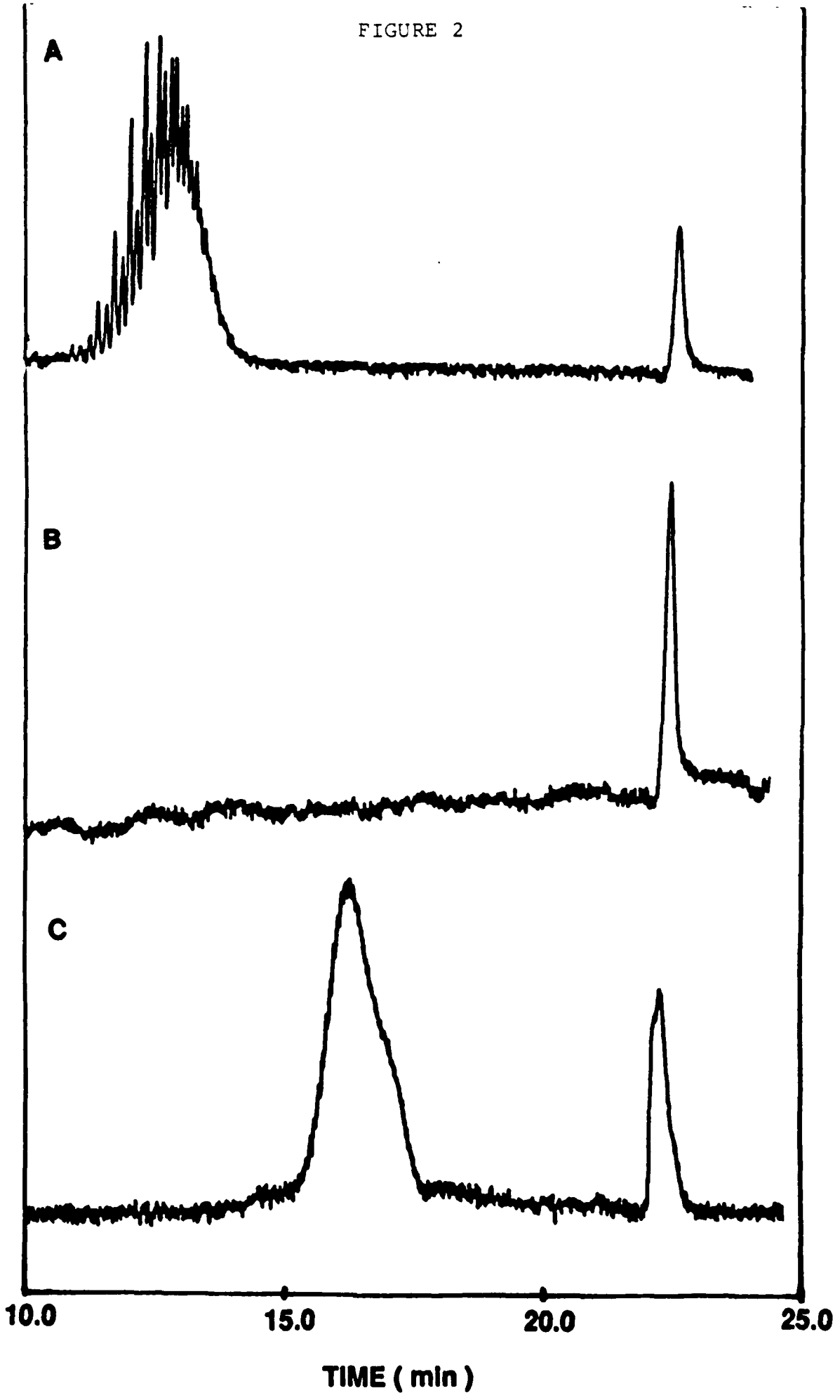
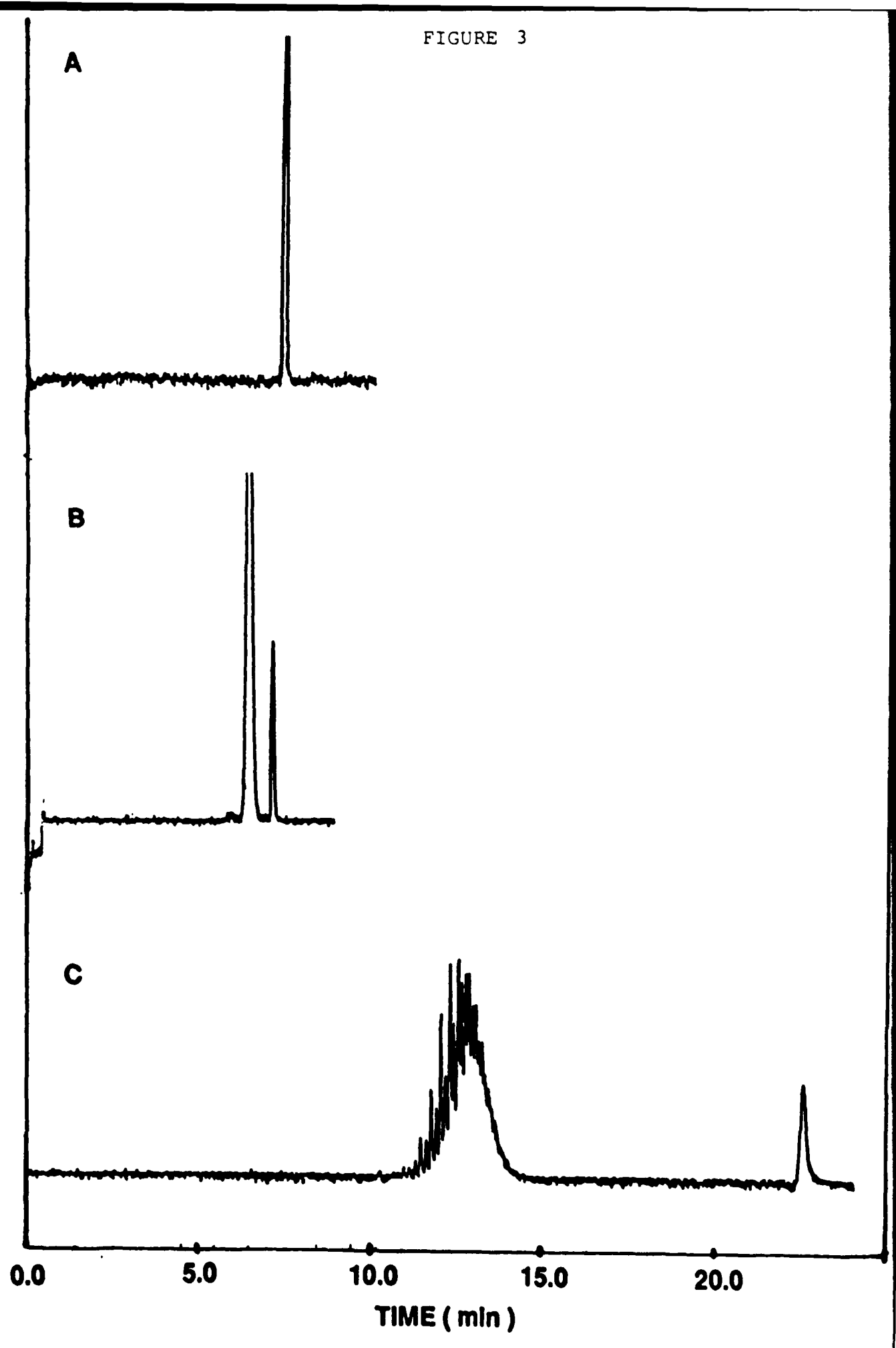


FIGURE 3



REFERENCES

1. (a) J.R. Mason and T.H. Morton, Physiol. Behav. 1982, 29, 709-714; (b) J.R. Mason and T.H. Morton, Tetrahedron 1984, 40, 483-492; (c) J.R. Mason, L. Clark, and T.H. Morton, Science 1984, 226, 1092-1094; (d) J.R. Mason, F.C. Leong, K.W. Plaxco, and T.H. Morton, J. Am. Chem. Soc. 1985, 107, 6075-6084; (e) J.R. Mason, K.K. Johri, and T.H. Morton, J. Chem. Ecol. 1987, 13, 1-18; (f) J.R. Mason, L. Clark, and T.H. Morton, Proc. N.Y. Acad. Sci. 1987, 510, 468-471.
2. G.R. Bear, J. Chromatogr. 1988, 459, 91-107.
3. (a) P.L. Desbene, B. Desmazieres, J.J. Basselier and A. Desbene-Monvernay, J. Chromatogr. 1989, 461, 305-313; (b) A. Aserin, N. Garti and M. Frenkel, J. Liquid Chromatogr. 19847 (8), 1545-1557.
4. J.M. Ernst, Anal. Chem. 1984, 56, 834-835.
5. A.Y. Tsygankov, Y.A. Motorin, A.D.Wolfson, D.B. Kirpotin and A.F. Ovlovsky, J. Chromatogr. 1989, 465, 325-329.
6. T. Okada, Anal. Chem. 1990, 62, 327-331.
7. T.L. Chester, J. Chromatogr. 1984, 299, 424-434.
8. B.E. Richter, J. High Resolu. Chromatogr. Chromatogr. Commun. 1985, 8, 297-300.
9. M. Ashrafkhorssani and L.T. Taylor, LC-GC.1990, 8(4), 314-320.
10. W.A. Pavelich and R.A. Livigni, J. Poly. Sci., Part C.1968, 21, 215-223.
11. L.H. Garcia-Rubio; J.F. McCregor; and A.E. Hamielec; In 'Polymer Characterization', C.D. Craver Ed., Amer. Chem. Soc., Washington, DC. 1983. pp. 311-344
12. J.C. Giddings; K.A. Graff; K.D. Caldwell and M.N. Myers; In 'Polymer Characterization', C.D. Craver Ed., Amer. Chem. Soc., Washington, DC. 1983. pp. 257-269.
13. A. Otsuki and H. Shiraishi, Anal. Chem. 1979, 51, 2329-2332.
14. H. Shiraishi; A. Otsuki and K. Fuwa, Bull. Chem. Soc., Jpn. 1982, 55, 1410-1415.
15. L.M. Nuwaysir, C.L. Wilkins and W.J. Simonsick, Jr., J.Amer. Soc. Mass Spectrom. 1990, 1, 66-71.
16. M.M. Siegel; R. Tsao; S. Oppenheimer and T.T. Chang, Anal. Chem. 1990, 62, 322-327.
17. J.W. Jorgenson and K.D. Lukacs, Science 1983, 222, 260-272.
18. B.L. Hogan and E.S. Yeung, J. Chromatogr. Sci. 1990, 28, 15-18.
19. J.W. Jorgenson and K.D. Lukacs, J. Chromatogr. 1981, 218, 209-216.

20. B. Nickerson and J.W. Jorgenson, J. High Resolu. Chromatogr. Chromatogr. Commun. 1988, 11, 533-534.

21. W.G. Kuhr and E.S. Yeung, Anal. Chem. 1988, 60, 2642-2646.

22. J.D. Rose, Jr. and J.W. Jorgenson, Anal. Chem. 1988, 60, 642-648.

23. K.D. Lukacs and J.W. Jorgenson, J. High Res. Chromatogr. Chromatogr. Commun. 1985, 8, 407-411.

24. W.G. Kuhr and E.S. Yeung, Anal. Chem. 1988, 60, 1832-1834.

25. J.W. Jorgenson and K.D. Lukacs, Anal. Chem. 1981, 53, 1298-1302.

26. R.A. Wallingford and A.G. Ewing, Anal. Chem. 1988, 60, 258-263.

27. K. Otsuka and S. Tearbe, J. Microcolumn Separation 1989, 1(3), 150-154.

28. B.B. VanOrman and C.L. McIntire, J. Microcolumn Separation 1989, 1(6), 289-293.

29. U.S. Patent 3,021,232 J.E. Pretka (to E.I. duPont de Nemours & Co.) Feb. 13, 1962.

30. Technical Report, Texaco Chemical Co. Texas USA.

31. P.D. Grossman, H.H. Lauer, S.E. Moring, D.E. Mead, M.F. Oldham, J. H. Nickel, J.R.P. Gouberg, A. Krever, D. H. Ranson and J.C. Colburn, Amer. Biotechnology Lab. 1990, 8(2), 35-43.

32. S.E. Moring, J.C. Colburn, P.D. Grossman and H.H. Lauer, LC.GC 1990 8(1), 34-46.

33. D.D. Perrin and B. Dempsey; In 'Buffers for pH and Metal Ion Control' Chapman and Hall, London. 1974

34. M.C. Roach and M.D. Harmony, Anal. Chem. 1987, 59, 411-415.

35. R.G. Carlson, K. Srinivasachar, R.S. Givens and B.K. Matuszewski, J. Org. Chem. 1986, 51, 3978-3987.

36. Dissociation Constants of Organic Bases in Aqueous Solutions. D.D. Perrin and D. Dalzell; Eds., Butterworths, London. 1965

37. A.S. Cohen, D.R. Najarian, A.Paulus, A. Guttman, J.A. Smith and B.L. Karger, Proc. Natl. Acad. Sci. 1988, 85, 9660-9663.